BIOCHEMICAL OXIDATION OF DAIRY WASTES

IV. Endogenous Respiration and Stability of Aerated Dairy Waste Sludge *

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In previous work, the rate and extent of conversion of milk solids to bacterial cells by the mixed culture of microorganisms which becomes dominant during aeration of milk waste (1)(3)(4) was determined. Chemical equations for the synthesis of cell tissue were derived from these data. From these equations and separate determinations of the rate of respiration, the rate at which oxygen is taken from the solution was calculated to be 0.075 lb. of oxygen per pound of milk solids per hour per 500 p.p.m. bacterial cells (0.4 p.p.m. oxygen per minute) during the period of rapid growth of cells.

The further digestion of these cells by their own (endogenous) respiration has a definite practical importance. If this respiration proceeds at a great enough rate, the cells can oxidize their own tissue (autodigestion of sludge) rapidly enough to keep the system in balance; that is, no sludge will accumulate. If the autodigestion is not sufficient, sludge will accumulate and disposal is necessary. The rate of endogenous respiration is of further interest because of the proposed partial-treatment process described previously, in which the cells would be discharged to a stream daily (2). The consequent drain on the oxygen supply of the stream would be directly related to this rate of endogenous respiration.

The experimental techniques used have been described previously (1)(6)(7)(9). An active culture containing about 500 p.p.m. bacterial solids was maintained by continuous feeding of 1,000 p.p.m. of skim milk solids into an aeration tank. The detention time was 20 hr. The rate of respiration was measured in a Warburg manometric apparatus (1) or by evolution of carbon dioxide (8). The latter method was especially valuable for experiments continued for several days.

Experimental Results

The rate of oxidation of skim milk was determined by the two methods on the same sludge and compared with the rate of endogenous respiration (Table I). Measurements were made after 6 hr. and calculated on an hourly basis. Three facts of importance are shown by these data, as follows:

TABLE I.—Oxidation of Skim Milk Solids *vs.* Endogenous Respiration

Method of Determination	Endogenous Respiration	Oxidation of Skim Milk Solids
Manometric: O_2 cons. (ml./l./hr.)[1]	3.2	32.1
Titrimetric: CO_2 prod. (ml./l./hr.)[1]	3.9	30.3

[1] Data averaged over initial 6 hr.; 1.0 ml. O_2 = 1.43 mg.; 1.0 ml. CO_2 = 1.96 mg.

1. The oxidation of milk solids proceeds at a rate of approximately 10 times that of endogenous respiration.

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2. The respiratory quotient (R.Q. = vol. CO_2/vol. O_2) is approximately 1.0, in agreement with previous results and the theoretical equations for the two reactions that have been developed (1). Thus, throughout this paper, Q_{CO_2} can be considered to be equal to Q_{O_2}, for they are equal on a volume basis if R.Q. = 1.0.

3. The relatively simple titrimetric determination of CO_2 produced gives data for determination of the rate of respiration that can be considered equivalent to Warburg manometric data for the present purposes.

The previous determinations of rate of oxidation were run for 6 hr., which was the time required to complete the rapid assimilation of milk solids into the cells. Over this period, unfed cells in control experiments maintained almost a constant rate of endogenous respiration (1). It was necessary to determine the rate over a longer time if the autodigestion in an aeration system with a holding time of 30 to 40 hr. was to be calculated. The Q_{CO_2} (microliters of carbon dioxide per milligram of cells per hour) was measured for 48 hr. (Figure 1). The amount of cells present initially was found to be 410 p.p.m. Measured amounts of gas produced per milliliter were calculated to the milligram of solids basis necessary for determining the rate of oxidation on an absolute basis (that is, Q_{CO_2}) by use of the equations previously established (4). The rapid assimilation reaction was at a high and relatively constant rate for 4 hr. During the 5th and 6th hours it was completed, and the rate fell to that of the unfed sample. For the following 42 hr., the rate was almost identical with that of the unfed control sample. The initial Q_{CO_2} of the latter sample was 10.3; over a period of 48 hr. it fell only to 4. Q_{O_2} and Q_{CO_2} values of 8 to 12 for endogenous respiration have been obtained in a number of other experiments over a 6-hr. period. It is considered, therefore, that a Q_{O_2} of 10 is an average value for this rate.

The oxygen consumption in parts per million per hour was calculated from these experiments, for parts per million of oxygen is the common unit of measurement for dissolved oxygen

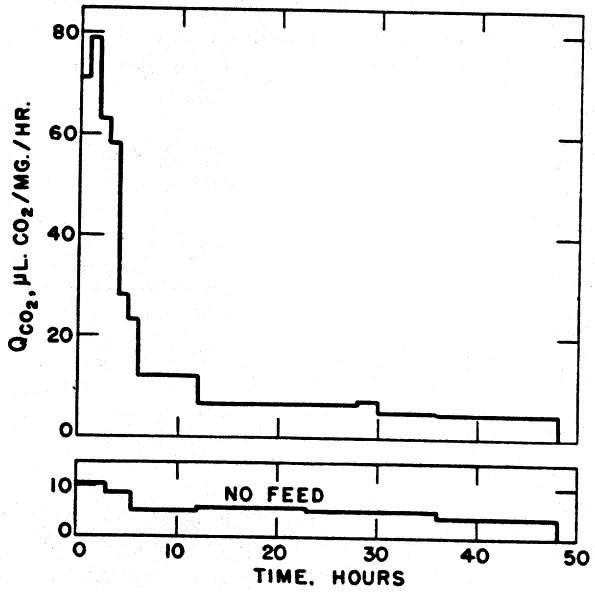

FIGURE 1.—Rate of removal of O_2 in oxidation of skim milk.

in sanitary engineering. The data in Figure 2 were obtained by direct manometric measurement of oxygen consumption. A maximum rate of 83 p.p.m. per hr. was obtained during the assimilation reaction. This value is much higher than that of 0.37 p.p.m. per min. or 22 p.p.m. per hr., which was determined by polarographic determination of oxygen removed (4). The greater rate may be due to the vigorous agitation of the culture in the manometric apparatus and the consequent lessened effect of diffusion through the liquid. Certainly, the rate measured in the present experiments can be considered comparable with the rate that would prevail in an aeration tank if oxygen were supplied fast enough. A second point of interest is the rate of oxygen uptake at the end of 18-hr. aeration, which was 7 p.p.m. per hr. It is readily seen that the air which should be supplied during the latter stages is much less than the maximum required for the first few hours.

These experiments were so designed that a convenient and accurate determination of the rate of oxidation could be made. They did not simulate the proposed partial-treatment process, for the final sludge was about twice as high in total solids as would be maintained by a fill-and-draw operation. An experiment was set up, therefore, in which 400 ml. of simulated waste (1,000 p.p.m. skim milk solids) were added to 100 ml. of active sludge that contained 380 p.p.m. solids. The rate of oxidation was determined by evolution of CO_2 and calculated to O_2 consumption per liter of mixed liquor (Figure 3). The addition of 4 volumes of a strong waste to 1 volume of a dilute sludge did not result in as high a rate of oxidation as was observed in the previous experiments, for there were not enough organisms to produce a maximum rate when the data are calculated on a volume basis. If the results were plotted as Q_{O_2}, the data would be comparable with those of Figure 2; the activity of the cells is high on a dry-weight basis.

The cumulative curve plotted against the ordinate on the right side of Figure 3 is significant, for it is in agreement with the theoretical analysis of the reactions published previously (4). The simulated waste, 400 ml. of a solution containing 1,000 p.p.m. skim milk solids, contained 400 × 0.88 (correction for ash and moisture), or 352

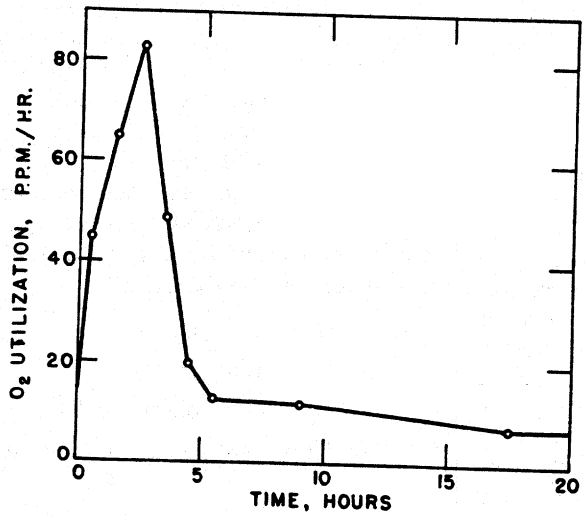

FIGURE 2.—Rate of aerobic oxidation of 1,000 p.p.m. skim milk solids.

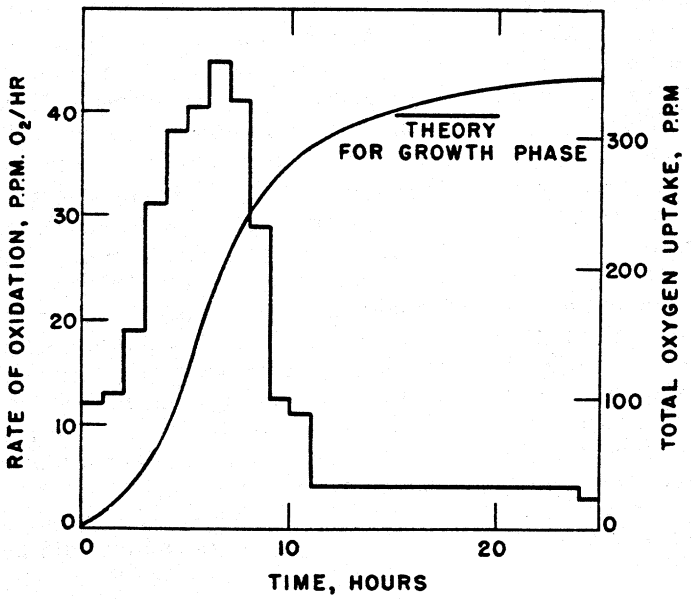

FIGURE 3.—Oxidation of 800 p.p.m. skim milk solids.

p.p.m. protein and lactose. According to the equations previously derived, the assimilation of this amount of skim milk would require $352:424::x:192$, $x = 159$ mg. O_2 per 500 ml., or 318 p.p.m. O_2. The rate of oxidation fell from that characteristic of the assimilation reaction to that of endogenous respiration when the cumulative oxygen consumption totaled 300 p.p.m., in excellent agreement with the value calculated from the earlier manometric experiments.

The absolute rate of the endogenous respiration for the whole 11- to 23-hr. period was 4 p.p.m. O_2 per hr.; for the final hour the rate was 3 p.p.m. O_2 per hr. Analysis for organisms present after 24 hr. showed there were 570 p.p.m. organic solids, yet the rate of oxidation was only 3 p.p.m. O_2 per hr. This low rate proves that the oxygen drain on a stream would be rather low and further justifies the proposal that a fill-and-draw aeration, with discharge of the whole effluent from the aerator would be an inexpensive and effective treatment for milk wastes (2) (5).

Discussion

The composition of the sludge organisms was established previously, and the following equation of endogenous respiration was derived (4):

$$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + NH_3 + 2\ H_2O$$
$$113 + 160 \rightarrow 220 + 17 + 36$$

The empirical formula $C_5H_7NO_2$ accounts for the organic constituents of the sludge. It has a formula weight of 113. The oxidation of one mole then requires 160 atomic weight units of O_2 and produces 220 units of CO_2. By use of these values and the following constants, the relative rate at which cells are consumed by their endogenous respiration can be calculated:

22.4 l. O_2 = 32 g. O_2

1 μl. O_2 = 1.43 μg. O_2

Q_{O_2} = μl. O_2 per mg. cells per hr.

A Q_{O_2} of 10 = 14.3 μg. O_2 per mg. cell tissue per hr.

According to the equation, 14.3 μg. $\times \frac{113}{160}$ = 10.2 μg. cell tissue oxidized

per hr. by 1 mg. of cells. But, 10.2 μg. = 0.0102 mg., or 1 per cent per hour. Therefore, a Q_{O_2} of 10 = 1 per cent of cell tissue oxidized endogenously each hour; a Q_{O_2} of 5 = 0.5 per cent, etc.

These calculations can be applied in practice as follows:

Assume that one volume per day of a waste containing 1,000 p.p.m. solids (640 p.p.m. 5-day B.O.D.) enters the treatment plant. The assimilation reaction will produce 500 p.p.m. of sludge. If a concentration of 2,500 p.p.m. of sludge, which has a Q_{O_2} of 10, is maintained in the system, 25 p.p.m. would be consumed by endogenous respiration hourly, or 500 p.p.m. in 20 hr. Such a system would be in balance and sludge would not accumulate.

The performance of an aeration system could be evaluated by the following determinations:

1. Chemical oxygen consumed and volume of waste entering.
2. Sludge solids (by oxygen consumed or direct determination).
3. Q_{CO_2} of sludge fed a simulated or actual waste.
4. Q_{CO_2} of unfed sludge.

Calculation of the solids balance from these measurements would give the performance of the treatment plant. For example, the oft-repeated statement that "this plant does not produce any sludge" could be checked. If the results showed an oxidative capacity appreciably lower than that required for complete oxidation, a reasonable possibility of occasional loss of sludge solids in the effluent would exist. If the rate was sufficient to keep the system balanced, the design and operation of the plant would be verified.

The data presented should also be evaluated on another basis. The primary purpose of plant waste treatment is to reduce the drain on the oxygen of the stream into which the effluent flows. According to the accepted standard of oxygen demand, the 5-day B.O.D., the fill-and-draw process reduces the pollution 75 to 80 per cent (2). The results (Figure 3) show that the endogenous respiration of the cells in the effluent which would be discharged to the stream is only 3 p.p.m. O_2 per hr. This rate is far lower than a comparison of the B.O.D. of the raw and treated waste would indicate. The raw waste has a maximum rate of oxidation of 40 to 45 p.p.m. O_2 per hr., and the treated waste less than 4 p.p.m. per hr., or a reduction of more than 90 per cent. It is probable that the greater reduction indicated by the rate of respiration is a more valid indication of the decrease in pollution than is given by the standard test. Carefully planned field experiments will be required to establish the validity of these latter ideas.

Summary

The rate of endogenous respiration (aerobic autodigestion) of the mixed culture of organisms that oxidizes dairy waste aerobically has been determined. The rate is low; the sludge produced by the oxidation of 1,000 p.p.m. skim milk solids consumes 3 to 4 p.p.m. O_2 per hr.

The same culture in the active-growth phase consumes oxygen at a rate of 40 to 45 p.p.m. O_2 per hr. The 10-fold difference between these rates is an important factor in the operation of existing plants and in the design of new plants.

The reduction in rate of oxidation brought about by conversion of milk solids to bacterial cells is much greater than the reduction in B.O.D. The theoretical and practical consequences of these differences are discussed.

Acknowledgment

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